

PI/RNASE Solution

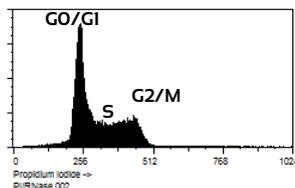
Reference	Size
PI/RNASE	200 test

PRODUCT DESCRIPTION

Propidium Iodide (PI) is the most commonly used dye for DNA and cell cycle analysis for flow cytometry. The PI binds to DNA by intercalating into the double stranded macromolecule. PI also binds to RNA, and is necessary to remove the RNA with a nucleases treatment (RNase) for optimal DNA resolution.

The quantification of the content DNA permits us to know the distribution of a cell population along the different phases of the cell cycle. In the analyses of a cell population by flow cytometry using dyes for DNA, the quantity of linked dye is proportional to the quantity of DNA.⁽¹⁾

The analyses of cell cycle by flow cytometry are represented in fluorescence intensity histograms for it probes specific of DNA. The cells of mammalian are characterized for having three populations or definite regions, cells in G2 and M phases of the cell cycle that have double DNA content of those in G0 and G1 phases, and a region correspond to cells in phase S.



The excitation of PI at 488 nm facilitates its use on all cytometers with argon ion lasers (most common flow cytometers).

Recommended usage: Immunostep's PI/RNase, is intended for analyses of cell cycle by flow cytometry.

Presentation: liquid

Storage Instruction: store PI/RNase between 2°C and 8°C. Do not use after expiration date stamped on vial.

Reagent provided: 200 test (500µl/test) of PI/RNase in 100 ml of PBS with 0,09% NaN₃ (sodium azide), pH 7.2.

Recommendation and warnings: This product contains sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop.

Before acquiring samples, adjust the discriminator (threshold) to minimize debris.

Staining cells protocol with PI/RNASE Solution. Flow Cytometry

1. Harvest the cells corresponding to 2×10^5 to 1×10^6 . Centrifuge the cells for 5 minutes at 300 xg, and remove the supernatant. Resuspend the pellet in the residual liquid.
2. Fix cell, add 200 µl of 70% ethanol by pipeting in the cell suspension slowly while vortexing.
3. Leave the cells in ethanol at 4°C for 30 min.
4. Wash cells once in 2 ml PBS + 2% BSA. Centrifuge the cells for 5 minutes at 300 xg, and remove the supernatant. Resuspend the pellet in the residual liquid.
5. Add 0,5 ml of propidium iodide solution (PI/RNase) to cell pellet and mix well. Incubate 15 minutes at room temperature before to analysis.
6. After incubation period, analyze by flow cytometry. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com)

Store samples at 4°C and protect from the light until analysed by flow cytometry.

Cells may be stored in 70% ethanol at -20 °C for several weeks prior to staining and flow cytometric analysis.

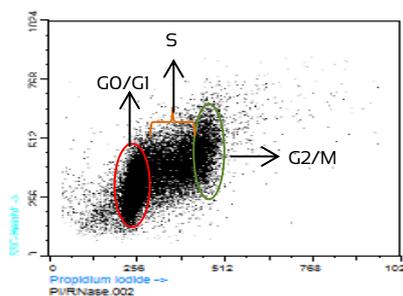


Figure 1. Jurkat cells (T-cell leukemia, human). DNA content analysis histogram (Top panel). Cell cycle analysis (Bottom panel)

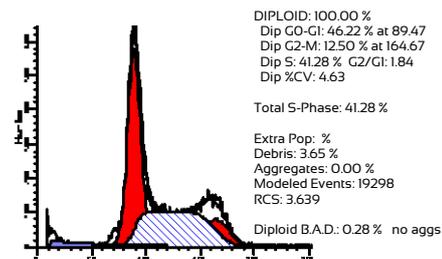


Figure 2. Jurkat cells (T-cell leukemia, human). Cells were analyzed using Modfit DNA analysis software.

Please, refer to <http://immunostep.com/content/31-support> for technical information.

WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

REFERENCES

1. Crowley LC, Chojnowski G, Waterhouse NJ. Measuring the DNA Content of Cells in Apoptosis and at Different Cell-Cycle Stages by Propidium Iodide Staining and Flow Cytometry. Cold Spring Harb Protoc Oct 03;2016(10):pdb prot087247.

MANUFACTURED BY



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